美葉蘇鐵疫病

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接受日期：民國 93 年 7 月19 日

摘　要

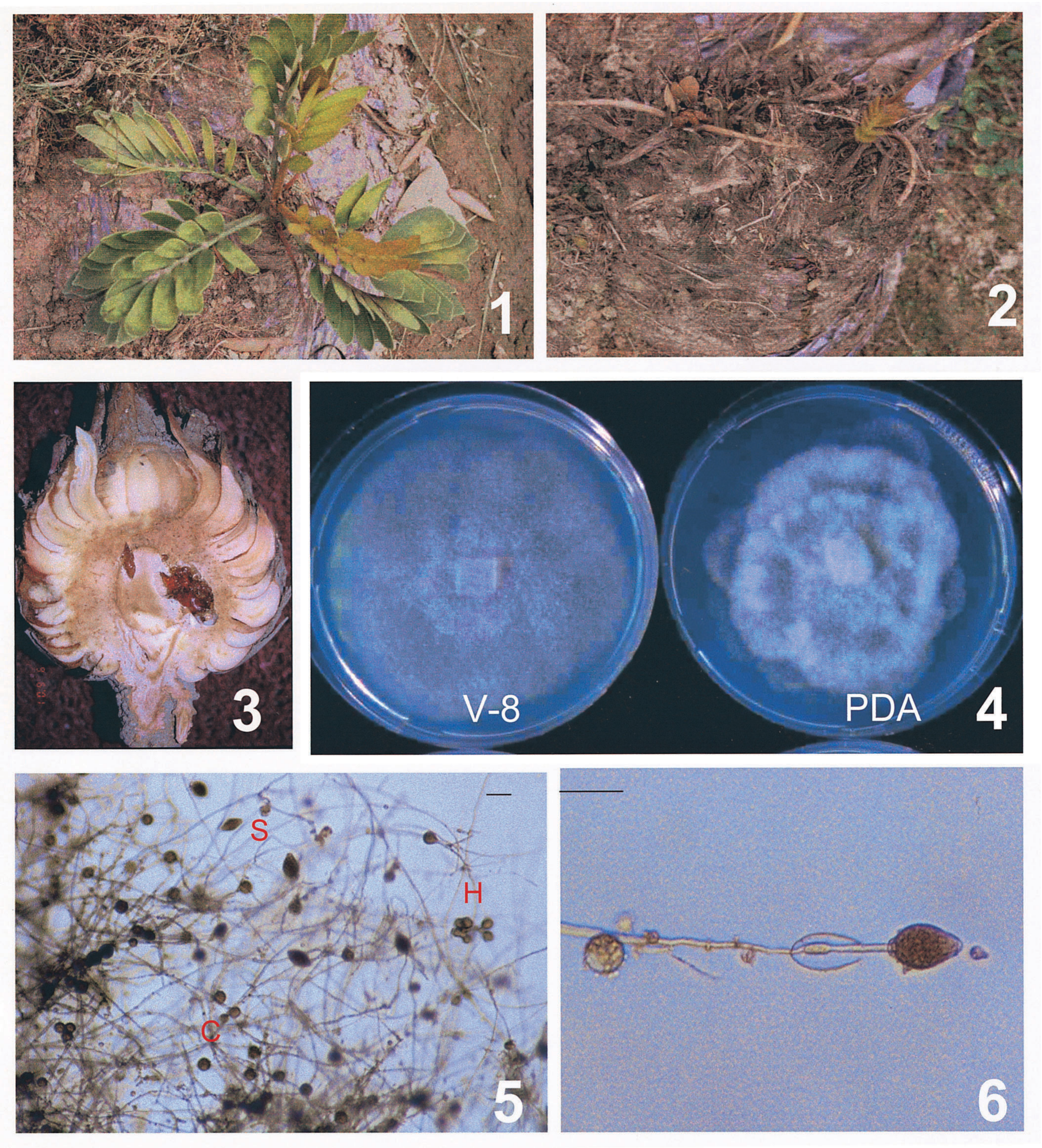
安寶貞、蔡志濃、王姻婷. 2004. 美葉蘇鐵疫病. 植病會刊 13:233-236.

西元二〇〇一年六月至七月間南投草屯蘇鐵育苗場種植之美葉蘇鐵 (*Zamia furfuracea*) 發生嚴重枯死情形。罹病植株葉片黃化、根系腐敗、莖部組織褐化，嚴重時全株枯萎死亡。經組織分離獲得3株疫病菌，將該疫病菌之游走子懸浮液接種蘇鐵幼苗根部，一個月後引起接種根部腐敗，並由罹病處分離得接種疫病菌，證明蘇鐵幼苗腐敗係因疫病菌引起。獲得之疫病菌的特性為：在馬鈴薯葡萄糖培養基(PDA) 上形成玫瑰花瓣狀之圖形；菌絲在 8-32℃下可生長，最適生長溫度為 24-28℃；胞囊須經土壤抽出液或礦物鹽液漂洗後才會形成，胞囊橢圓形、長橢圓形，不具乳突 (non-papilate) 亦不脫落，胞囊釋放游走子後會再生內生或串生胞囊；形成珊瑚狀菌絲膨脹體及厚膜胞子；配對型為 A^1，可刺激 A^2 型之 *Phytophthora parasitica* 形成卵胞子，但本身無卵胞子產生；經鑑定為 *Phytophthora cinnamomi* Rands。*P. cinnamomi* 危害蘇鐵為世界首度報導。

關鍵詞：蘇鐵疫病、*Phytophthora cinnamomi*

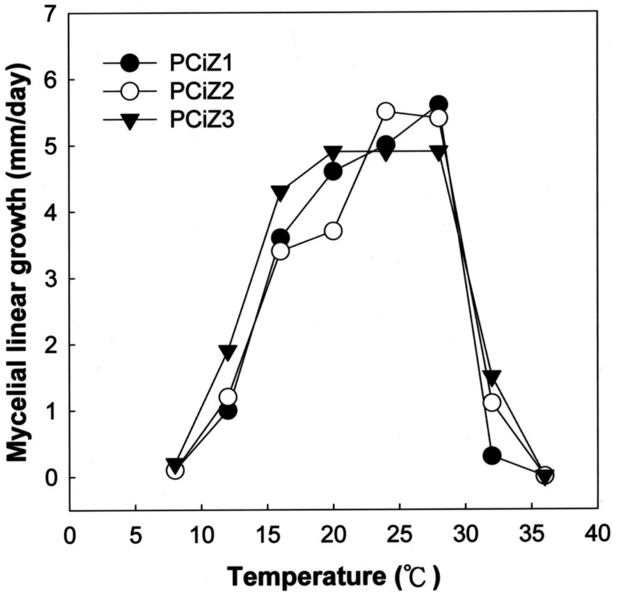
美葉蘇鐵(*Zamia furfuracea* L.)，又稱闊葉蘇鐵，英名 Sago Cycas，原產於墨西哥，為蘇鐵科美葉蘇鐵屬之常綠小灌木。美葉蘇鐵得名於葉子長得又大又美麗，近年來引進臺灣作為盆栽觀賞用。西元2001 年6-7 月間南投草屯蘇鐵育苗場種植之美葉蘇鐵苗發生嚴重黃萎枯死情形。得病植株的複葉出現不均勻褪色情形 (圖一)，嚴重者全葉黃化、生育停頓(圖二)，而終至全株死亡；罹病植株的根系嚴重腐敗，將球莖組織縱向切開後，患部呈現淡褐色，並伴有流膠現象 (圖三)，隨切開時間加長而患部顏色漸深呈褐色，但患部與健康部位之分界不明顯。將罹病蘇鐵採回，患部組織切成 7×7×2 mm^3 小塊，經 0.5 % NaClO 溶液表面消毒 30 sec，放置於 5 % CV-8A+AMP [7] 之半選擇性培養基之培養皿 (直徑9cm) 內。於分離後第二天開始，疫病菌菌絲即陸續自病組織長出，共獲得 3 株疫病菌 (PCiZ1-PCiZ3)，將疫病菌經單游走子純化後，移植於新鮮之 5 % V-8A (5 % V-8 vegetable juice agar, 含 5 % V-8 Vegetable juice 與 0.02 % $CaCO_3$ 混合後，加入 2 % Bacto agar)上。利用 Hwang 等 [6] 研發的方法，讓供試菌株產生胞囊 (sporangia)，並配製游走子懸浮液 (zoospore suspension) (每毫升約含 10^4 zoospores)。將健康蘇鐵幼苗根系去除土壤後，浸於游走子懸浮液中 24 hr，再種回滅菌過之土壤中。接種 10 天後將接種蘇鐵苗自盆中取出，有部份鬚根已經腐敗，將未完全腐敗之根切成 1cm 長小段，放置於含 5 % CV-8A+AMP 之半選擇性培養基上，可以分離獲得相同的接種疫病菌。接種一個月後，將植株莖部切開，可見與根系接觸之莖基部已經開始褐變腐敗，亦可分得相同的接種疫病菌。而接種蒸餾水之對照處理於試驗期間並無腐敗情形發生。

自蘇鐵所分離之疫病菌可在 8-32℃生長，最適生長溫度為24-28℃，於室溫下 (25-28℃) 在 5 % CV-8A 上生長形成之菌落無特殊花紋，具氣生菌絲；在 PDA (馬鈴薯葡萄糖瓊脂) 上形成不太顯著之玫瑰花瓣狀花紋 (圖四)。菌絲在 5 % V-8A 上不會形成胞囊，但將新鮮菌絲塊置於土壤抽出液中(soil extract solution, 田土／蒸餾水 (V/V) = 1/1)，或利用 Hwang 等 [6] 研發的方法，將菌絲塊經礦物鹽液漂洗處理後，可形成胞囊 (圖五)。每一胞囊梗 (sporangiophore) 的頂端可著生 1-3 胞囊，大部分情形僅著生一個胞囊。胞囊橢圓形、長橢圓形或檸檬形，不具乳突 (non-papilate)，亦不脫落 (non-deciduous)，胞囊釋放游走子後會再生內生或串生胞囊 (inter- or nested proliferate sporangia) (圖六)；胞囊(不包括再生胞囊) 大小為42.5-65.0 ×27.5-35 μm 平均為 53.5×32.1 μm；胞囊長寬比 (L/B) 為 1.21-2.7，平均 1.67。蘇鐵疫病菌在 V-8A 上或水中形成厚膜胞子 (chlamydospores) 與珊瑚狀菌絲膨脹體 (coralloid



圖一～六、*Phytophthora cinnamomi* 引起美葉蘇鐵之病徵與病菌形態。外觀病徵(圖一)、嚴重罹病株病徵(圖二)及罹病莖部之縱切面(圖三)；病菌在5% V-8A與PDA 生長六天的菌落形態(圖四)；及該菌在經礦物鹽水洗後形成之胞囊、厚膜胞子及菌絲膨脹體形態(圖五)與再生胞囊之形態(圖六)。(線長=50 μm)

Figs 1-6. Disease symptoms of *Zamia furfuracea* caused by *Phytophthora cinnamomi* and morphologies of the pathogen. A moderately infected plant (Fig. 1) and a serious infected plant (Fig. 2); The longitudinal section of a diseased stem with brown discoloration and gummosis (Fig. 3); The colony patterns of the isolate 21160 grown on 5%CV-8A and PDA for 6 days under room temperature (Fig. 4); Sporangia (S), chlamydospores(C), and hyphal swellings(H) in liquid condition (Fig. 5) and a internal proliferating sporangium (Fig. 6). (Bar=50 μm)



圖七、危害美葉蘇鐵之疫病菌 *Phytophthora cinnamomi* 在 5% CV-8 瓊脂上於不同溫度下的每日生長情形。
Fig. 7. Daily growth curve of 3 isolates of *Phytophthora cinnamomi* isolated from *Zamia furfuracea* on 5%CV-8 agar at different temperatures (6 days).

hyphal swellings)(圖五)，厚膜胞子大小 25-35 μm，平均為 31 μm。該類菌株在單獨培養時於 20 % V8-A 不形成卵胞子，但與 *Phytophthora parasitica* Dastur A^2 配對型之標準菌株 P731 對峙培養後會產生卵胞子，因此均為 A^1 配對型 (A^1 mating type)。利用柯氏研發之夾膜法 (7)，將蘇鐵疫病菌株與 A^2 菌株 P731 隔薄膜(polycarbomate nuclepore membrane, 孔徑 0.2 μm) 對峙培養後，測試菌株本身不會產生卵胞子 (selfing oospores)，但可刺激 A^2 菌株形成卵胞子，因此供試菌株屬 Sexuality type S^2 (8)。比較分類文獻 (10,11)，雖然該菌無卵胞子產生，但依據胞囊形態與珊瑚狀之菌絲膨脹體，顯示此疫病菌應為 *Phytophthora cinnamomi* Rands，並屬於 Waterhouse 分類中的第六群 (Phytophthora Group VI) (11)。

依據 Zentmyer (1980) 報告 (12)，*P. cinnamomi* 是疫病菌中寄主範圍最多且分布最廣泛的疫病菌，該菌遍佈歐、亞、美、澳等洲，寄主範圍超過千種以上。該菌主要危害植物根系，造成根部腐敗與植株生長衰弱，嚴重時導致植株死亡。在台灣，除蘇鐵外，有記錄的 *P. cinnamomi* 的寄主共有 10 屬 12 種植物 (1,3,5)，包括鳳梨、茶花、小葉雞納樹、大葉雞納樹、杜鵑、酪梨、樟樹、土肉桂、台灣肖楠 (4)、柑橘 (2)、四季海棠 (3) 及進口百合 (1)，且其存在天然林的土壤中 (natural forest soil)(9)。其中受害最嚴重的作物為酪梨，為該經濟作物產業的限制因子 (未發表)。此次發現疫病菌 *P. cinnamomi* 危害美葉蘇鐵為世界首度報導。

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ABSTRACT

Ann, P. J. [1,2], Tsai. J. N. [1], and Wang, I. T. [1] 2004. *Phytophthora cinnamomi* on *Zamia furfuracea* in Taiwan. Plant Pathol. Bull. 13:233-236. ([1.] Plant Pathology Division, Agricultural Research Institute, Wufeng, Taichung, Taiwan; [2.] Corresponding author, E-mail: pjann@wufeng.tari.gov.tw; Fax: +886-4-23338162)

Serious seedling decline of *Zamia furfuracea* was found at Tsaotuan, Nantow in the summer of 2001. The leaves of the affected seedlings became smaller and yellow at the early stage and their root systems were necrotic and seriously rotted. The internal tissues of affected stems turned brown and soft with gummosis. The diseased plants grew slowly and died eventually. Three isolates of *Phytophthora* were isolated and all of them caused disease symptoms similar to those appearing in the field on the inoculated young seedlings. The same fungi were reisolated from the inoculated diseased seedlings. The isolated *Phytophthora* grew on V-8 agar at temperature ranging from 8-32℃ with the optimum temperatures of 24-28℃. The fungal colonies showed rosette pattern on PDA plates. Hyphal swellings were spherical, irregular to coralloid, clustered or single. Sporangia were not formed on agar plates but were produced abundantly in soil extract solution or washed with mineral solution. Sporangia, 42.5-(53.5)-65.0 × 27.5-(32.1)-35 μm, were non-papillate and non-deciduous, and displayed nested and extended proliferation in water. The ratios of L/B were 1.21-(1.67)-2.7. Chlamydospores, 25-(31)-35 μm, were spherical and thin-walled. All of the three *Phytophthora* isolates belonged to A^1 mating type. None of them form oospores themselves when paired with A^2 type of *P. parasitica*. However, they were able to stimulate A^2 to initiate sexual reproduction. The *Phytophthora* was identified as *Phytophthora cinnamomi* Rands according to Waterhouse' key. This is the first report of Phytophthora disease of *Zamia furfuracea* in Taiwan as well as in the world.

Key words : *Zamia furfuracea*, *Phytophthora cinnamomi*